FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
PF98PCTSEQ/dln	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	
09/936676	
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE
PCT/FR00/00622	15 MAR 2000 (12.03.00)
PRIORITY DATE CLAIMED	
15 MAR 1999 (15.03.99)	

TITLE OF INVENTION

BACTERIAL MEMBRANE FRACTIONS WITH ADJUVANT EFFECT

APPLICANT(S) FOR DO/EO/US

Christine LIBON, Nathalie CORVAIA, Thien Ngoc N'GUYEN, Alain BECK, Jean-Yves BONNEFOY

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☒ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Sequence listing paper copy
Sequence listing disk copy
Statement by Attorney under 37 CFR §1.821(f)

531 Rec'd PC... 14 SEP 2001

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/936676	PCT/FR00/00622	PF98PCTSEQ/dln

21. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :	CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO $860.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	38 - 20 =	18	x $18.00	$324.00
Independent claims	1 - 3 =	0	x $80.00	$0.00
Multiple Dependent Claims (check if applicable).			☐	$0.00
TOTAL OF ABOVE CALCULATIONS			=	$1,184.00
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).**			☐	$0.00
SUBTOTAL			=	$1,184.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	$0.00
TOTAL NATIONAL FEE			=	$1,184.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	$0.00
TOTAL FEES ENCLOSED			=	$1,184.00
			Amount to be: refunded	$
			charged	$

☒ A check in the amount of **$1,184.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **8-3220** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

G. Patrick Sage
THE FIRM OF HUESCHEN AND SAGE
500 Columbia Plaza
350 East Michigan Ave.
Kalamazoo MI, 49007

G. Patrick Sage
SIGNATURE

G. Patrick Sage
NAME

37,710
REGISTRATION NUMBER

September 14, 2001
DATE

PF98PCTSEQ/dln

* * * * *

Applicant : Christine LIBON, et al.

Title : BACTERIAL MEMBRANE FRACTIONS WITH ADJUVANT EFFECT

* * * * *

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

A soon as a Serial Number and Filing Date have been accorded the above-identified national phase application, kindly amend as follows:

IN THE CLAIMS: Kindly cancel all of the Claims, 1 through 33, and replace by Claims 34 through 71 as provided herewith.

REMARKS

The present application is a national phase filing of PCT/FR00/00622

Applicants have cancelled all of the originally-filed Claims, 1 through 33. New Claims 34 through 71 have been added to better encompass the full scope and breadth of the invention notwithstanding Applicants' belief that the Claims would have been allowable as originally filed. Accordingly, Applicants assert that no Claims have been narrowed within the meaning of *Festo*.

Entry of the new Claims and early and favorable action on the merits of this application are respectfully solicited.

Respectfully submitted,

THE FIRM OF HUESCHEN AND SAGE

By: 
G. PATRICK SAGE

Dated: September 13, 2001
Customer No.: 25,666
500 Columbia Plaza
350 East Michigan Ave.
Kalamazoo, MI 49007
(616) 382-0030

Enclosure: Postal Card Receipt
Claims 34 through 71

We Claim:

- 34 -

The use of a *Klebsiella pneumonia* membrane fraction combined with an antigen or hapten for the preparation of a pharmaceutical composition intended to orient the immune response toward a Th1 type and/or mixed Th1/Th2 type response directed against the antigen or hapten, in which response the Th1 response is close to or greater than the Th2 type response.

- 35 -

The use of Claim 34, wherein the membrane fraction comprises at least membrane fractions of two different bacterial strains.

- 36 -

The use of Claim 34, wherein the membrane fraction is prepared by a method comprising the following steps:

a) culture of the bacteria in a culture medium allowing their growth followed by centrifugation of the culture;

b) where appropriate, deactivation of the lytic enzymes of the bacterial pellet obtained in step a), followed by centrifugation of the suspension obtained;

c) extraction and removal of nonmembrane proteins and of nucleic acids from the pellet obtained in step a) or b) by at least one cycle of washing the pellet in an extraction solution;

d) digestion of the membrane pellet obtained in step c) in the presence of protease enzymes, followed by centrifugation;

e) at least one cycle of washing of the pellet obtained in step d) in physiological saline and/or in distilled water; and

f) ultrasonication of the pellet obtained in step e).

- 37 -

The use of Claim 34, wherein the membrane fraction is prepared by a method comprising the following steps:

a) culture of the bacteria in a culture medium allowing their growth, followed, where appropriate, by centrifugation;

b) freezing of the culture medium or of the pellet obtained in step a) followed by thawing and drying of the cells;

c) removal, by means of a DNase, of the nucleic acids from the dry cells obtained in step b) which have been resuspended;

d) grinding of the cells obtained in step c) and clarification of the suspension obtained;

e) precipitation, in an acid medium, of the suspension obtained in step d) and removal of the pellet;

f) neutralization of the supernatant obtained in step e) containing the membrane suspension, followed by dialysis and concentration of the membrane suspension; and

g) sterilization of the concentrated membrane suspension obtained in step f).

- 38 -

The use of Claim 34, wherein the antigen or hapten is chosen from the antigens or haptens specific to an infectious agent or from the antigens associated with tumor cells.

- 39 -

The use of Claim 38, wherein the antigen or hapten is chosen from peptides, lipopeptides, polysaccharides, oligosaccharides, nucleic acids, lipids or any compound capable of specifically directing the Th1 type and/or mixed Th1/Th2

type immune response against an antigen or hapten specific to an infectious agent or an antigen associated with a tumor cell.

- 40 -

The use of Claim 34, wherein the antigen or hapten is coupled or mixed with the membrane fraction.

- 41 -

The use of Claim 34, wherein the antigen or hapten is covalently coupled with a supporting peptide to form a complex capable of specifically binding to mammalian serum albumin.

- 42 -

The use of Claim 41, wherein the supporting peptide is a peptide fragment derived from streptococcal G protein.

- 43 -

The use of Claim 41, wherein the complex is prepared by genetic recombination.

- 44 -

The use of Claim 41, wherein the antigen, hapten or complex is covalently coupled with at least one of the compounds contained in the membrane fraction.

- 45 -

The use of Claim 44, wherein the covalent coupling is a coupling carried out by chemical synthesis.

- 46 -

The use of Claim 45, wherein one or more linking elements are introduced into at least one of the compounds contained in the membrane fraction and/or in the antigen, hapten or complex to facilitate the chemical coupling.

- 47 -

The use of Claim 46, wherein the linking element introduced is an amino acid.

- 48 -

The use of Claim 44, wherein the coupling between the antigen, hapten or complex and at least one of the compounds contained in the membrane fraction, is carried out by genetic recombination when the antigen, hapten or complex and the membrane compound are of a peptide nature.

- 49 -

The use of Claim 34, wherein the pharmaceutical composition comprises an agent which makes it possible to carry the membrane fraction associated with the antigen, hapten or complex in a form which makes it possible to enhance its stability and/or its immunogenecity.

- 50 -

The use of Claim 49, wherein the agent is an oil-in-water or water-in-oil type emulsion.

- 51 -

The use of Claim 49, wherein the agent is a particle of the liposome, microsphere or nanosphere type or any type of structure allowing the

encapsulation and the presentation in particulate form of the membrane fraction associated with the antigen, hapten or complex.

- 52 -

The use of Claim 49, wherein the agent is chosen from aluminum salts, calcium salts, compounds of plant origin such as Quil A or saponin, or compounds of bacterial origin such as cholera, pertussis or tetanus toxoid or thermolabile E. coli toxin.

- 53 -

The use of Claim 34, wherein the pharmaceutical composition comprises an agent which makes it possible to regulate the immune response induced by the membrane fraction associated with the antigen, hapten or complex.

- 54 -

The use of Claim 53, wherein the regulatory agent is chosen from cytokines, growth factors, hormones or cellular components such as nucleic acids, a protein of the family of heat shock proteins or ribosomes.

- 55 -

The use of Claim 34 for the preparation of a pharmaceutical composition intended for the prevention or treatment of infectious diseases or cancers.

- 56 -

The use of Claim 55, wherein the infectious disease is of viral, bacterial, fungal or parasitic origin.

- 57 -

The use of Claim 56 for the preparation of a pharmaceutical composition intended for the prevention or treatment of paramyxovirus infections.

- 58 -

The use of Claim 57, wherein the paramyxovirus is a respiratory syncytial virus.

- 59 -

The use of Claim 58, wherein the antigen associated with the membrane fraction comprises the peptide G2Na of SEQ ID No. 4 or one of its homologs whose sequence exhibits a degree of identity of at least 80% with SEQ ID No. 4.

- 60 -

The use of Claim 59, wherein the peptide G2Na or one of its homologs is covalently coupled with a C-terminal fragment (BB) of the streptococcal G protein to form a complex capable of binding to mammalian serum albumin.

- 61 -

The use of Claim 57, wherein the paramyxovirus is a parainfluenzae virus.

- 62 -

A pharmaceutical composition comprising a membrane fraction prepared by the method of Claim 36 and an antigen or hapten associated with the membrane fraction.

- 63 -

A pharmaceutical composition comprising a membrane fraction prepared by the method of Claim 37 and an antigen or hapten associated with the membrane fraction.

- 64 -

The pharmaceutical composition of Claim 62, wherein the antigen is chosen from paramyxovirus peptide fragments.

- 65 -

The pharmaceutical composition of Claim 63, wherein the antigen is chosen from paramyxovirus peptide fragments.

- 66 -

The pharmaceutical composition of Claim 64, wherein the paramyxovirus is a respiratory syncytial virus or a parainfluenzae virus.

- 67 -

The pharmaceutical composition of Claim 65, wherein the paramyxovirus is a respiratory syncytial virus or a parainfluenzae virus.

- 68 -

The pharmaceutical composition of Claim 66, wherein the antigen associated with the membrane fraction comprises the peptide G2Na of SEQ ID No. 4 of the respiratory syncytial virus or a peptide whose sequence exhibits a degree of identity of at least 80% with SEQ ID No. 4.

- 69 -

The pharmaceutical composition of Claim 67, wherein the antigen associated with the membrane fraction comprises the peptide G2Na of SEQ ID No. 4 of the respiratory syncytial virus or a peptide whose sequence exhibits a degree of identity of at least 80% with SEQ ID No. 4.

- 70 -

The pharmaceutical composition of Claim 68, wherein the peptide G2Na, or one of it homologs, is covalently coupled with a C-terminal fragment (BB) of the streptococcal G protein to form a complex capable of binding to mammalian serum albumin.

- 71 -

The pharmaceutical composition of Claim 69, wherein the peptide G2Na, or one of it homologs, is covalently coupled with a C-terminal fragment (BB) of the streptococcal G protein to form a complex capable of binding to mammalian serum albumin.

BACTERIAL MEMBRANE FRACTIONS WITH ADJUVANT EFFECT

The present invention relates to the use of a membrane fraction of gram-negative bacteria, in particular of Klebsiella *pneumoniae*, combined with an antigen or hapten, for the preparation of a pharmaceutical composition intended for orienting the immune response toward a Th1 type and/or mixed Th1/Th2 type response directed against said antigen or hapten. This invention comprises, in addition, methods for the preparation of said membrane fractions and the pharmaceutical compositions containing them and their applications to the prevention and treatment of infectious diseases, in particular infections caused by paramyxoviruses such as RSV, and cancers, in particular those whose tumors are associated with tumor antigens.

Vaccination is an effective means for preventing or reducing in particular infections. The success of vaccination campaigns in this field has made it possible to extend the concept of vaccines to the fields of autoimmune diseases, cancer and fertility. On the other hand, vaccinating antigens alone are not always capable of inducing a rapid and sustained antibody response, which requires the presence of adjuvants, that is to say compounds which help (from the latin *adjuvare*: to help) them to induce such responses.

Adjuvants constitute a group of varied compounds with respect to their structure and their origin. There are thus, inter alia, in this category water-in-oil (incomplete Freund's adjuvant) or oil-in-water emulsions, compounds of bacterial origin such as lipopolysaccharide derivatives from Gram-negative bacteria and aluminum salts. Currently, only aluminum salts are used in humans as adjuvant for vaccine preparations.

The development of an antibody response directed against an antigen requires a series of complex events. It involves cells presenting the antigen, regulatory T lymphocytes (Th for T "helper"), and antibody-producing B lymphocytes. Two types of Th lymphocytes may be distinguished according to the profile of cytokines produced: type 1 Th lymphocytes producing IFN-γ and IL-2 and promoting the formation of IgG2a in mice, and type 2 Th lymphocytes producing IL-4, IL-5 and IL-10 with formation of IgG1 in mice (Mosmann, T.R. and Sad S. Immunol. Today 1996, 17:138). Moreover, it has been shown that, for the same given antigen, it is the adjuvant which orients toward the predominant isotype during the antibody response (Toellner K.-M. et al. J. Exp. Med. 1998, 187:1193). Thus, it is known that aluminum salts, such as Alhydrogel, induce, in mice, an essentially Th2 type response and promote the formation of IgG1 or even of IgE (Allison A.C. In Vaccine design - The role of cytokine networks Vol. 293, 1-9 Plenum Press 1997), which can pose problems in subjects with an allergic predisposition. Furthermore, according to the therapeutic target envisaged, a Th1 or mixed (Th1/Th2) type response may be desired.

Thus, there is currently a need to have available novel adjuvants capable of inducing an immune response of the Th1 or mixed (Th1/Th2) type, preferably a mixed Th1/Th2 response for which the Th1 response is close to or greater than the Th2 response.

Surprisingly, the authors of the present invention have demonstrated particular properties of the membrane fraction of a gram-negative bacterium Klebsiella *pneumoniae* (called FMKp), in particular membrane fractions obtained by methods as described below in the examples. The authors have indeed discovered that said membrane fraction FMKp, combined with an antigen, not only had the capacity to increase the antibody response directed against said antigen but also had the capacity

to reorient the cytokine response toward a Th1/Th2 profile, thus corresponding to the particular adjuvant activity sought, this being regardless of the mode of administration of said membrane fractions.

Thus, the subject of the present invention is the use of a membrane fraction of gram-negative bacteria, in particular of Klebsiella *pneumoniae*, combined with an antigen or hapten for orienting the immune response toward a Th1 type and/or mixed Th1/Th2 type response directed against said antigen or hapten, or for the preparation of a pharmaceutical composition intended for orienting the immune response toward a Th1 type and/or mixed Th1/Th2 type response directed against said antigen or hapten.

By orientation of the immune response toward a Th1 and/or mixed Th1/Th2 type response, there is preferred in particular orientation of the immune response which promotes the induction of a Th1 response relative to the Th1/Th2 response obtained with the alum adjuvant.

By orientation of the immune response toward a Th1 and/or mixed Th1/Th2 type response, there is more particularly preferred an orientation of the immune response which increases the titer of IgG2a antibodies directed against the associated antigen by a factor of at least 10, preferably of at least 25, 50 and 100 relative to the IgG2a titer obtained with the alum adjuvant.

In a particularly preferred manner, the immune response is oriented toward a Th1 and/or mixed Th1/Th2 type response in which the Th1 response is close to or greater than the Th2 response. The expression "close to" will be understood to mean a response which, when expressed as titer of IgG2a antibodies directed against the associated antigen, is at least equal to 0.5 times, preferably at least equal to 0.75 times, the

titer of IgG1 antibody directed against said antigen, with a titer of IgG antibody directed against the associated antigen close to or greater than the titer of IgG antibody directed against the associated antigen obtained with the alum or Freund's adjuvant.

The invention also relates to the use according to the invention, characterized in that the membrane fraction comprises at least membrane fractions of two different strains of bacteria.

The expression membrane fraction of a bacterium is understood to mean, in the present invention, any purified or partially purified membrane fraction or extract obtained from a culture of said bacterium and whose method of preparation comprises at least a step of lysing the bacteria obtained after culture and a step of separating the fraction containing the membranes of said bacteria from the total lysate obtained after the lysis step, in particular by centrifugation or filtration.

The expression membrane fraction of the bacterium when said bacterium is Klebsiella *pneumoniae* is also understood to mean, in the present invention, protein P40, an active fraction of the membrane fraction of Klebsiella *pneumoniae*, having an amino acid sequence SEQ ID No. 2, or one of its fragments.

According to the invention, the membrane fractions may be prepared according to methods known to a person skilled in the art, such as for example the method described by Haeuw J.F. et al. (Eur. J. Biochem, 255, 446-454, 1998).

According to a particular embodiment, the invention relates to a use according to the invention, characterized in that the membrane fraction is prepared by a method comprising the following steps:

a) culture of said bacteria in a culture medium allowing their growth followed by centrifugation of said culture;

b) where appropriate, deactivation of the lytic enzymes of the bacterial pellet obtained in step a), followed by centrifugation of the suspension obtained;

c) extraction and removal of nonmembrane proteins and of nucleic acids from the pellet obtained in step a) or b) by at least one cycle of washing the pellet in an extraction solution;

d) digestion of the membrane pellet obtained in step c) in the presence of proteolytic enzymes, followed by centrifugation;

e) at least one cycle of washing of the pellet obtained in step d) in physiological saline and/or in distilled water; and

f) ultrasonication of the pellet obtained in step e).

Step b) of deactivation of the lytic enzymes of the bacterial pellet obtained in step a) may be carried out by any known methods of deactivation of enzymes, such as in particular by heating the resuspended bacterial pellet at a temperature preferably of close to 100°C or by adding an inhibitor of the activity of these enzymes.

Step c) of extraction and removal of the nonmembrane proteins and of the nucleic acids from the pellet obtained in step a) or b) may be carried out, for example, by at least one cycle of washing of the pellet in an extraction solution corresponding to the addition of a hypertonic solution (extraction solution), preferably a saline solution having a molarity of close to 1 M, followed, after a contact period which is sufficient for the desired effect, by centrifugation of the suspension obtained and removal of the supernatant obtained after said centrifugation, it being possible for this washing cycle to be repeated several times.

Step d) of digestion of the membrane pellet obtained in step c) may be carried out in the presence of a solution of proteolytic enzymes such as, for example, trypsin, chymotrypsin, or any known enzyme with proteolytic activity, the reaction conditions, pH of the solution, temperature and duration of the reaction, being preferably adjusted to the optimum conditions for the activity of the chosen enzyme(s), followed by centrifugation, it being possible for this digestion cycle to be repeated several times with the same enzyme, the same combination of enzymes or with a different enzyme for each digestion cycle performed.

Step e) of washing the pellet obtained in step d) is carried out by taking up the pellet in physiological saline or in distilled water followed, after a sufficient period of contact, by centrifugation, it being possible for this washing cycle to be repeated several times.

Finally step f) of ultrasonication of the pellet is intended in particular to disintegrate and homogenize the membrane fraction obtained at the end of step e). The ultrasonication conditions (duration and intensity) will be determined by persons skilled in the art, for example, according to the quantity of membrane fraction to be treated.

According to another particular embodiment, the invention relates to a use according to the invention, characterized in that the membrane fraction is prepared by a method comprising the following steps:

a) culture of said bacteria in a culture medium allowing their growth, followed, where appropriate, by centrifugation;
b) freezing of the culture medium or of the pellet obtained in step a) followed by thawing and drying of the cells;

c) removal, by means of a DNase, of the nucleic acids from the dry cells obtained in step b) which have been resuspended;

d) grinding of the cells obtained in step c) and clarification of the suspension obtained;

e) precipitation, in an acid medium, of the suspension obtained in step d) and removal of the pellet;

f) neutralization of the supernatant obtained in step e) containing the membrane suspension, followed by dialysis and concentration of the membrane suspension; and

g) sterilization of the concentrated membrane suspension obtained in step f).

The thawing conditions in step b) of the method below will of course be determined by persons skilled in the art according to the initial quantity of pellet to be treated, preferably carried out at 4°C for at least 48 hours for the equivalent of 1 kg of dry cells.

In step c), the removal of the nucleic acids is carried out, for example, by the addition of a DNase, at a final concentration of 5 mg/ml of a cell suspension at a concentration equivalent to 5% of dry cells.

The grinding of the cells obtained in step c) may be carried out by means of any system or apparatus known to a person skilled in the art for grinding cells, such as presses or preferably such as grinding in a Manton Gaulinet loop for 30 minutes.

The clarification of the suspension obtained after grinding may be carried out by means of any system or apparatus known to a person skilled in the art for the clarification of ground products of bacterial cells such as the Sharpless system.

Step e) of precipitation in acid medium of the suspension obtained in step d) may be carried out, for example, with acetic acid. The precipitation is followed by the removal of the pellet by means of a Sharpless-type system and by recovering of the supernatant.

Step f) consists in a step in which the supernatant, obtained after precipitation in acid medium, is neutralized, diluted, dialyzed and then concentrated.

Finally, the last step consists in a step of sterilizing the membrane fraction concentrate obtained in the preceding step such as, for example, by heating at 121°C for about 35 minutes.

The invention particularly relates to the use according to the invention, characterized in that the membrane fraction is the Klebsiella *pneumoniae* protein P40 having the sequence SEQ ID No. 2, or one of these fragments.

The expression protein P40 fragment is understood to mean in particular any fragment having an amino acid sequence contained in the amino acid sequence of protein P40 capable of increasing a nonspecific immune response and/or capable of inducing an antitumor immune response, and comprising at least 5 amino acids, preferably at least 10 amino acids and more preferably at least 15 amino acids.

Of course, said protein P40, or its fragments, may be obtained by chemical synthesis or in the form of recombinant peptides.

The invention particularly relates to the use according to the invention, characterized in that said antigen or hapten is chosen from the antigens or haptens specific to an infectious agent, such as a virus, a bacterium, a

fungus or a parasite, or from the antigens associated with tumor cells.

According to the invention, said antigens or haptens are preferably chosen from peptides, lipopeptides, polysaccharides, oligosaccharides, nucleic acids, lipids or any compound capable of specifically directing the immune response toward a Th1 type and/or mixed Th1/Th2 type response against an antigen or hapten specific to an infectious agent or an antigen associated with a tumor cell.

Of course, said antigen or hapten, when it is of a peptide nature, may be obtained by chemical or recombinant synthesis.

The methods for preparing recombinant peptides are nowadays well known to persons skilled in the art and will not be developed in the present description. Among the cells which may be used for the production of these recombinant peptides, there may of course be mentioned bacterial cells (Olins P.O. and Lee S.C., 1993, Recent advances in heterologous gene expression in E. coli Curr. Op. Biotechnology 4:520-525), but also yeast cells (Buckholz R.G., 1993, Yeast Systems for the Expression of Heterologous Gene Products. Curr. Op. Biotechnology 4:538-542), as well as animal cells, in particular mammalian cell cultures (Edwards C.P. and Aruffo A., 1993, Current applications of COS cell based transient expression systems. Curr. Op. Biotechnology 4, 558-563) but also insect cells in which methods may be used involving for example baculoviruses (Luckow V.A., 1993, Baculovirus systems for the expression of human gene products. Curr. Op. Biotechnology 4, 564-572).

The invention comprises, in addition, the use according to the invention, characterized in that said antigen or hapten is coupled or mixed with said membrane fraction,

in particular covalently coupled with at least one of the compounds contained in the membrane fraction.

In a preferred embodiment, the invention comprises the use according to the invention, characterized in that said antigen or hapten is covalently coupled with a supporting peptide to form a complex capable of binding specifically to mammalian serum albumin, preferably said supporting peptide is a peptide fragment derived from streptococcal G protein, in particular the C-terminal fragment called BB.

Of course, said complex may be prepared by genetic recombination.

The chimeric or hybrid complex may be produced by recombinant DNA techniques by insertion or addition of a sequence encoding said antigen or hapten of a protein nature to a DNA sequence encoding said peptide fragment of the streptococcal G protein.

The methods for the synthesis of hybrid molecules include the methods used in genetic engineering for constructing hybrid polynucleotides encoding the desired polypeptide sequences. Reference may be advantageously made, for example, to the technique for producing genes encoding fusion proteins which is described by D.V. Goeddel (Gene expression technology, Methods in Enzymology, Vol. 185, 3-187, 1990).

According to the present invention, the covalent coupling may be carried out by chemical synthesis. In a particular embodiment of the invention, it will be possible for one or more linking elements to be introduced into at least one of the compounds contained in the membrane fraction and/or in said antigen or hapten to facilitate the chemical coupling.

Preferably, said linking element introduced is an amino acid.

According to the invention, it is possible to introduce one or more linking elements, in particular amino acids, to facilitate the coupling reactions between a compound of the membrane fraction, and said antigen or hapten. The covalent coupling between said compound of the membrane fraction and said antigen or hapten according to the invention may be achieved at the N- or C-terminal end of said compound of the membrane fraction or of said antigen or hapten, if the latter are for example of a peptide nature. The bifunctional reagents allowing the coupling will be determined according to the end which is chosen for the coupling and the nature of said antigen or hapten to be coupled.

The invention also comprises the use according to the invention, characterized in that the coupling between said antigen, hapten or complex and at least one of the compounds contained in the membrane fraction is carried out by genetic recombination when said antigen, hapten or complex and said membrane compound are of a peptide nature.

The coupling between said antigen, hapten or complex, and at least one of the compounds contained in the membrane fraction may indeed be carried out by genetic recombination. It will be possible, for example, before extracting its membrane fraction, to transform said gram-negative bacterium beforehand with a vector containing a nucleic construct encoding an antigen of interest or said complex, such that the bacterium thus transformed expresses the antigen of interest or said complex attached to the membrane or anchored in the membrane of said bacterium. Such methods for expressing recombinant proteins attached to the membrane are well known and require, for example, the presence of a

specific regulatory sequence, such as a signal peptide-type sequence.

The subject of the invention is also the use according to the invention, characterized in that the pharmaceutical composition comprises, in addition, an agent which makes it possible to carry said membrane fraction associated with said antigen, hapten or complex in a form which makes it possible to enhance its stability and/or its immunogenicity, such as in the form of an oil-in-water or water-in-oil type emulsion, or in the form of a particle of the liposome, microsphere or nanosphere type or any type of structure allowing the encapsation and the presentation, in particulate form, of said membrane fraction associated with said antigen, hapten or complex.

The invention also relates to the use according to the invention, characterized in that said agent is chosen from aluminum salts, calcium salts, compounds of plant origin such as Quil A or saponin, or compounds of bacterial origin such as the derivatives of cholera, pertussis or tetanus toxoid or of the E. coli thermolabile toxin.

Also included in the present invention is the use according to the invention, characterized in that the pharmaceutical composition comprises, in addition, an agent which makes it possible to regulate the immune response induced by said membrane fraction combined with said antigen, hapten or complex.

Among said regulatory agents, cytokines, growth factors, hormones or cellular components such as nucleic acids, a protein of the family of heat shock proteins or ribosomes are in particular preferred.

The subject of the invention is also the use according to the invention, for the preparation of a

pharmaceutical composition intended for the prevention or treatment of infectious diseases of viral, bacterial, fungal or parasitic origin, or for the prevention or treatment of cancers, in particular cancers in which the tumors are associated with tumor antigens.

Among said infectious diseases of viral origin, the infectious diseases caused by paramyxoviruses, in particular by the parainfluenzae virus and more preferably by the respiratory syncytial virus (RSV) are particularly preferred.

In a particular embodiment, the use according to the invention is characterized in that said antigen associated with the membrane fraction comprises the peptide G2Na, a fragment of the G protein of the virus having an amino acid sequence SEQ ID No. 4, a peptide homologous to G2Na whose sequence exhibits at least 80%, preferably 90%, 95% and 99% identity, after alignment with the sequence SEQ ID No. 4, or the peptide G2Na or one of its homologs, covalently coupled with a C-terminal fragment (BB) of the streptococcal G protein to form a complex capable of binding to mammalian serum albumin, peptide BB as described in the documents Power et al., 1997 (Virology, 230, 155-166) and WO 96/14416.

The expression "percentage, degree or level of identity" between two nucleic acid or amino acid sequences for the purposes of the present invention is understood to mean a percentage of identical nucleotides or amino acid residues between the two sequences to be compared, obtained after the best alignment, this percentage being purely statistical and the differences between the two sequences being randomly distributed over their full length. The sequence comparisons between two nucleic acid or amino acid sequences are traditionally carried out by

comparing these sequences after having aligned them in an optimum manner, said comparison being carried out by segment or by "comparison window" to identify and compare the local regions of sequence similarity. The optimum alignment of the sequences for the comparison may be carried out either manually or by means of the local homology algorithm by Smith and Waterman (1981) [Ad. App. Math. 2:482], by means of the local homology algorithm by Neddleman and Wunsch (1970) [J. Mol. Biol. 48:443], by means of the method of search for similarity by Pearson and Lipman (1988) [Proc. Natl. Acad. Sci. USA 85:2444], by means of computer software using these algorithms (GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI, or by the comparison software packages BLAST N or BLAST P).

The percentage identity between two nucleic acid or amino acid sequences is determined by counting these two sequences optimally aligned by the comparison window in which the region of the nucleic acid or amino acid sequence to be compared may comprise additions or deletions relative to the reference sequence for an optimum alignment between these two sequences. The percentage identity is calculated by determining the number of identical positions for which the nucleotide or the amino acid residue is identical between the two sequences, by dividing this number of identical positions by the total number of positions in the comparison window and by multiplying the result obtained by 100 in order to obtain the percentage identity between these two sequences.

For example, it will be possible to use the BLAST program, "BLAST 2 sequences", available on the site http://www.ncbi.nlm.nih.gov/gorf/bl2.html, the parameters used being those given by default (in particular for the parameters "open gap penaltie": 5, and "extension gap penaltie": 2; the template chosen

being for example the template "BLOSUM 62" proposed by the program), the percentage identity between the two sequences to be compared being calculated directly by the program.

In another aspect, the invention relates to a method for preparing a membrane fraction of gram-negative bacteria, in particular Klebsiella *pneumoniae*, characterized in that it comprises the following steps:

a) culture of said bacteria in a culture medium allowing their growth followed by centrifugation of said culture;

b) where appropriate, deactivation of the lytic enzymes of the bacterial pellet obtained in step a), followed by centrifugation of the suspension obtained;

c) extraction and removal of nonmembrane proteins and of nucleic acids from the pellet obtained in step a) or b) by at least one cycle of washing the pellet in an extraction solution;

d) digestion of the membrane pellet obtained in step c) in the presence of protease enzymes, followed by centrifugation;

e) at least one cycle of washing of the pellet obtained in step d) in physiological saline and/or in distilled water; and

f) ultrasonication of the pellet obtained in step e).

The invention also comprises the method for preparing a membrane fraction of gram-negative bacteria, in particular Klebsiella *pneumoniae*, characterized in that it comprises the following steps:

a) culture of said bacteria in a culture medium allowing their growth, followed, where appropriate, by centrifugation;

b) freezing of the culture medium or of the pellet obtained in step a) followed by thawing and drying of the cells;

c) removal, by means of a DNase, of the nucleic acids from the dry cells obtained in step b) which have been resuspended;

d) grinding of the cells obtained in step c) and clarification of the suspension obtained;

e) precipitation, in an acid medium, of the suspension obtained in step d) and removal of the pellet;

f) neutralization of the supernatant obtained in step e) containing the membrane suspension, followed by dialysis and concentration of the membrane suspension; and

g) sterilization of the concentrated membrane suspension obtained in step f).

The membrane fractions capable of being obtained by said methods indeed form part of the invention.

The content of proteoglycan of the membrane fractions capable of being obtained by said methods, an active ingredient of FMKp, represented by the sum of the contents of galactose and of protein, is preferably between:

- for galactose: between 1.2 g/l and 3.4 g/l;
- for the proteins: between 7.5 g/l and 14.9 g/l.

More preferably, this content will be:

- for galactose: between 1.6 g/l and 2.6 g/l;
- for the proteins: between 9.3 g/l and 11.7 g/l.

The invention relates, in addition, to the pharmaceutical compositions comprising a membrane fraction capable of being obtained by the methods according to the invention, preferably, said

pharmaceutical compositions comprise, in addition, an antigen, a hapten or a complex, as defined above, associated with said membrane fraction, such as in particular viral antigens or complexes specific to paramyxoviruses, or the antigens associated with tumor cells.

Of course, said pharmaceutical compositions according to the invention may comprise, in addition, the agents such as the vehicles and the regulatory agents defined above.

In a preferred embodiment, the pharmaceutical composition according to the invention is characterized in that said antigen associated with the membrane fraction comprises the peptide G2Na having the sequence SEQ ID No. 4 of the respiratory syncytial virus, one of its homologs as defined above, said peptide G2Na, or one of its homologs, covalently coupled with a C-terminal fragment (BB) of the streptococcal G protein to form a complex capable of binding to mammalian serum albumin.

The legend to the figures and examples which follow are intended to illustrate the invention without in any way limiting the scope thereof.

Legend to the figures:

Figure 1: BBG2Na adjuvanted with FMKp - dose-response study (serum anti-G2Na IgG titers).
* $p < 0.05$ (compared with the PBS group).
Figure 2: BBG2Na adjuvanted with FMKp - anti-G2Na IgG1 and IgG2a titers.
Figure 3: BBG2Na adjuvanted with FMKp - protection study.
Figure 4: Adjuvant effect of FMKp toward Immugrip (influenza vaccine).

* $p < 0.05$ compared with the nonadjuvanted group ("0") on the same day of sample collection.

Example 1: Production of the membrane fraction of *K. pneumoniae* (FMKp)

Method No. 1

The extraction of the K. pneumoniae I145 membranes from the centrifugation pellet of the step is preferably preceded by a step of destroying the lytic enzymes of the cellular components obtained in the pellet, for example by heating the latter at 100°C, optionally after redissolving in solution.

The actual extraction of the membranes from the centrifugation pellet is preferably carried out by treating the cellular components of the pellet, after optional destruction of the lytic enzymes, with a saline solution, for example 1 M sodium chloride, once or several times, followed by centrifugation, preferably at 20,000 g, of the suspension obtained, the supernatant from this centrifugation, which is eliminated, contains nonmembrane impurities such as proteins and nucleic acids, while the pellet contains the membranes.

After separation of the saline solution containing the impurities, the membranes are digested in the presence of proteolytic enzymes, preferably trypsin and chymotrypsin, in solution at pH 8 at 37°C for 4 hours.

After digestion, the solution is homogenized by ultrasonication. The product thus obtained constitutes the membrane fraction called FMKp.

The supernatant obtained is again centrifuged under the same conditions, preferably at 140,000 g.

Preparation of the membrane glycopeptides

This fraction is prepared from the pellet obtained by centrifugation at 40,000 g for 20 minutes. Said pellet is resuspended in physiological saline and then this suspension is heated for 10 minutes at 100°C on a boiling water bath to inactivate the lytic enzymes. After cooling, the medium is centrifuged for 30 min at 20,000 g. The pellet obtained is extracted twice with 1M NaCl in order to eliminate the proteins and the nucleic acids. The membranes are recovered by centrifugation for 30 minutes at 20,000 g.

They are then subjected to digestion with trypsin at pH 8 and at 37°C for 4 hours and then with chymotrypsin under the same conditions.

The membranes are then recovered by centrifugation at 2000 g for 30 minutes, washed with physiological saline and then with distilled water and are subjected to disintegration by ultrasound for 15 minutes.

Method No. 2

After thawing at +4°C for 48 h minimum, 1 kg of dry K. pneumoniae cells is resuspended in solution at 5% dry cells. The DNase is added at 5 mg/l. Grinding in a Manton Gaulin loop is then carried out for 30 min, followed by clarification on SHARPLES at 50 l/h, followed by precipitation with acetic acid at pH = 4.2 + 0.1 for 30 min. The pellet is removed (SHARPLES at 25 l/h) and the supernatant is neutralized, diluted to twice the initial volume with osmosed water. Constant-volume dialysis is then carried out on PUF 100 up to 800 Ωcm, followed by concentration of the membrane suspension (MS) thus obtained, to 11 l/kg of dry cells. The MS is then autoclaved at +121°C for 35 min and preserved at +4°C for 6 weeks.

Characteristics of the FMKp

By definition, the content of proteoglycan, an active ingredient of FMKp, is equal to the sum of the contents of galactose and of proteins.

- Galactose: on average 2.2 g/l
- Proteins: on average 10.5 g/l.

Example 2: Adjuvant effect of FMKp on a recombinant protein, BBG2Na

BBG2Na is a recombinant protein produced in *E. coli*. It consists of the peptide G2Na having the sequence SEQ ID No. 4, the fragment of the G protein of the respiratory syncytial virus (RSV) type A extending from residue 130 to residue 230, fused with BB, a fragment of the streptococcal G protein, having the capacity to bind to serum albumin. BBG2Na is an anti-RSV vaccine (Power U. Virology 1997, 230:155-166).

BALB/c mice receive 2 subcutaneous injections of 20 µg of BBG2Na and various quantities of FMKp. Blood samples are collected on D28 and the serum antibody titers are determined by ELISA with G2Na in solid phase. The results obtained are illustrated by figure 1. Surprisingly, they show that FMKp significantly increases the anti-G2Na IgG response; the anti-G2Na IgG titer reached is similar to those induced by alum or Freund's adjuvant. The effect is dose-dependant: it is observed from 5 µg of FMKp, is maximum from 50 µg of FMKp and remains stable with 100 µg of FMKp. FMKp is therefore a potential adjuvant for BBG2Na.

To know the effect of FMKp on the orientation of the immune response, in terms of Th1/Th2 response, the anti-G2Na IgG1 and IgG2a titers were determined on sera obtained as specified above. The results (figure 2) show that, surprisingly, FMKp is capable of modifying

the anti-G2Na IgG1/IgG2a ratio, in contrast to that which is observed with alum, for which the predominant isotype is IgG1. This profile is close to that induced by Freund's adjuvant. This indicates that FMKp may be used as immunity adjuvant to induce a mixed (Th1/Th2) type response.

The animals immunized as described above received a viral challenge by the nasal route with 10^5 $TCID_{50}$ of RSV-A. This was carried out 3 weeks after the last immunization. Five days after the viral challenge, the animals were sacrificed and the lungs removed in order to determine the RSV-A titers. The results (figure 3) show that the animals which received BBG2Na adjuvanted with FMKp are protected against an RSV-A challenge.

In conclusion, FMKp makes it possible to reorient the antibody response without affecting the capacity to protect mice against an RSV-A challenge.

Example 3: Adjuvant effect of FMKp on an inactivated virus (influenza vaccine)

BALB/c mice receive a single injection of 0.01 µg of Immugrip™ (influenza vaccine marketed by Laboratoires INAVA), and various quantities of FMKp. The products are coadministered. The injection is performed subcutaneously at D0. Blood samples are collected at D7, D14 and D21. The anti-Immugrip serum IgG antibody titer is determined by ELISA with Immugrip at 2 µg/ml in solid phase. The results presented (figure 4) show that FMKp significantly increases the anti-Immugrip antibody titer, this being from the lowest dose of FMKp, namely 0.1 µg. The adjuvant effect is dose-dependant. It is observed, interestingly, that the presence of FMKp induces the generation of an earlier antibody response, obtained from D7, compared with the nonadjuvanted Immugrip control. This effect is not

obtained with the reference adjuvant, complete Freund's adjuvant (CFA).

CLAIMS

1. The use of a Klebsiella *pneumoniae* membrane fraction combined with an antigen or hapten for the preparation of a pharmaceutical composition intended to orient the immune response toward a Th1 type and/or mixed Th1/Th2 type response directed against said antigen or hapten, in which response the Th1 response is close to or greater than the Th2 type response.

2. The use as claimed in claim 1, characterized in that the membrane fraction comprises at least membrane fractions of two different bacterial strains.

3. The use as claimed in either of claims 1 and 2, characterized in that the membrane fraction is prepared by a method comprising the following steps:
   a) culture of said bacteria in a culture medium allowing their growth followed by centrifugation of said culture;
   b) where appropriate, deactivation of the lytic enzymes of the bacterial pellet obtained in step a), followed by centrifugation of the suspension obtained;
   c) extraction and removal of nonmembrane proteins and of nucleic acids from the pellet obtained in step a) or b) by at least one cycle of washing the pellet in an extraction solution;
   d) digestion of the membrane pellet obtained in step c) in the presence of protease enzymes, followed by centrifugation;
   e) at least one cycle of washing of the pellet obtained in step d) in physiological saline and/or in distilled water; and

f) ultrasonication of the pellet obtained in step e).

4. The use as claimed in either of claims 1 and 2, characterized in that the membrane fraction is prepared by a method comprising the following steps:

a) culture of said bacteria in a culture medium allowing their growth, followed, where appropriate, by centrifugation;

b) freezing of the culture medium or of the pellet obtained in step a) followed by thawing and drying of the cells;

c) removal, by means of a DNase, of the nucleic acids from the dry cells obtained in step b) which have been resuspended;

d) grinding of the cells obtained in step c) and clarification of the suspension obtained;

e) precipitation, in an acid medium, of the suspension obtained in step d) and removal of the pellet;

f) neutralization of the supernatant obtained in step e) containing the membrane suspension, followed by dialysis and concentration of the membrane suspension; and

g) sterilization of the concentrated membrane suspension obtained in step f).

5. The use as claimed in one of claims 1 to 4, characterized in that said antigen or hapten is chosen from the antigens or haptens specific to an infectious agent or from the antigens associated with tumor cells.

6. The use as claimed in claim 5, characterized in that said antigen or hapten is chosen from peptides, lipopeptides, polysaccharides, oligosaccharides, nucleic acids, lipids or any

compound capable of specifically directing the Th1 type and/or mixed Th1/Th2 type immune response against an antigen or hapten specific to an infectious agent or an antigen associated with a tumor cell.

7. The use as claimed in one of claims 1 to 6, characterized in that said antigen or hapten is coupled or mixed with said membrane fraction.

8. The use as claimed in one of claims 1 to 7, characterized in that said antigen or hapten is covalently coupled with a supporting peptide to form a complex capable of specifically binding to mammalian serum albumin.

9. The use as claimed in claim 8, characterized in that said supporting peptide is a peptide fragment derived from streptococcal G protein.

10. The use as claimed in either of claims 8 and 9, characterized in that said complex is prepared by genetic recombination.

11. The use as claimed in one of claims 7 to 10, characterized in that said antigen, hapten or complex is covalently coupled with at least one of the compounds contained in the membrane fraction.

12. The use as claimed in claim 11, characterized in that the covalent coupling is a coupling carried out by chemical synthesis.

13. The use as claimed in claim 12, characterized in that there are introduced one or more linking elements into at least one of the compounds contained in the membrane fraction and/or in said

antigen, hapten or complex to facilitate the chemical coupling.

14. The use as claimed in claim 13, characterized in that said linking element introduced is an amino acid.

15. The use as claimed in claim 11, characterized in that the coupling between said antigen, hapten or complex and at least one of the compounds contained in the membrane fraction is carried out by genetic recombination when said antigen, hapten or complex and said membrane compound are of a peptide nature.

16. The use as claimed in one of claims 1 to 15, characterized in that the pharmaceutical composition comprises, in addition, an agent which makes it possible to carry said membrane fraction associated with said antigen, hapten or complex in a form which makes it possible to enhance its stability and/or its immunogenecity.

17. The use as claimed in claim 16, characterized in that said agent is an oil-in-water or water-in-oil type emulsion.

18. The use as claimed in claim 16, characterized in that said agent is a particle of the liposome, microsphere or nanosphere type or any type of structure allowing the encapsulation and the presentation in particulate form of said membrane fraction associated with said antigen, hapten or complex.

19. The use as claimed in claim 16, characterized in that said agent is chosen from aluminum salts, calcium salts, compounds of plant origin such as

Quil A or saponin, or compounds of bacterial origin such as cholera, pertussis or tetanus toxoid or thermolabile E. coli toxin.

20. The use as claimed in claims 1 to 19, characterized in that the pharmaceutical composition comprises, in addition, an agent which makes it possible to regulate the immune response induced by said membrane fraction associated with said antigen, hapten or complex.

21. The use as claimed in claim 20, characterized in that said regulatory agent is chosen from cytokines, growth factors, hormones or cellular components such as nucleic acids, a protein of the family of heat shock proteins or ribosomes.

22. The use as claimed in one of claims 1 to 21, for the preparation of a pharmaceutical composition intended for the prevention or treatment of infectious diseases or cancers.

23. The use as claimed in claim 22, characterized in that the infectious disease is of viral, bacterial, fungal or parasitic origin.

24. The use as claimed in claim 23, for the preparation of a pharmaceutical composition intended for the prevention or treatment of paramyxovirus infections.

25. The use as claimed in claim 24, characterized in that the paramyxovirus is a respiratory syncytial virus.

26. The use as claimed in claim 25, characterized in that said antigen associated with the membrane

fraction comprises the peptide G2Na having the sequence SEQ ID No. 4 or one of its homologs whose sequence exhibits a degree of identity of at least 80% with the sequence SEQ ID No. 4.

27. The use as claimed in claim 26, characterized in that said peptide G2Na or one of its homologs is covalently coupled with a C-terminal fragment (BB) of the streptococcal G protein to form a complex capable of binding to mammalian serum albumin.

28. The use as claimed in claim 24, characterized in that the paramyxovirus is a parainfluenzae virus.

29. A pharmaceutical composition, characterized in that it comprises a membrane fraction prepared by the method as defined in either of claims 3 and 4, and an antigen or hapten associated with said membrane fraction.

30. The pharmaceutical composition as claimed in claim 29, characterized in that said antigen is chosen from paramyxovirus peptide fragments.

31. The pharmaceutical composition as claimed in claim 30, characterized in that the paramyxovirus is a respiratory syncytial virus or a parainfluenzae virus.

32. The pharmaceutical composition as claimed in claim 31, characterized in that said antigen associated with the membrane fraction comprises the peptide G2Na having the sequence SEQ ID No. 4 of the respiratory syncytial virus or a peptide whose sequence exhibits a degree of identity of at least 80% with the sequence SEQ ID No. 4.

33. The pharmaceutical composition as claimed in claim 32, characterized in that said peptide G2Na or one of its homologs is covalently coupled with a C-terminal fragment (BB) of the streptococcal G protein to form a complex capable of binding to mammalian serum albumin.

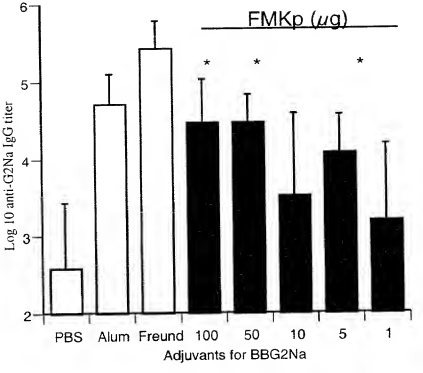

FIGURE 1

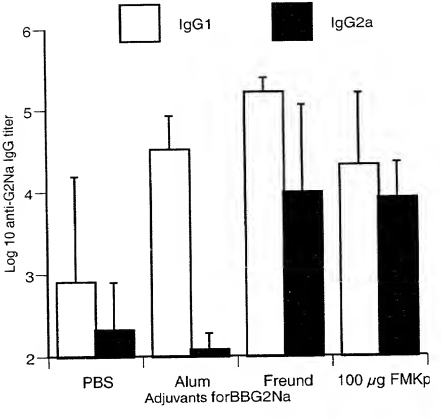

FIGURE 2

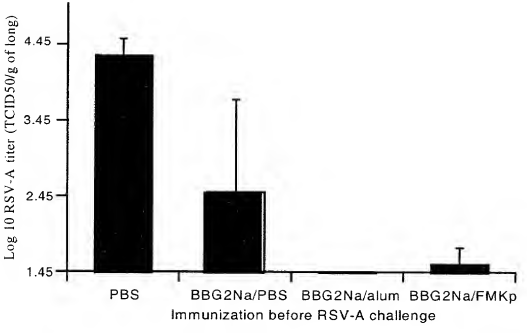

FIGURE 3

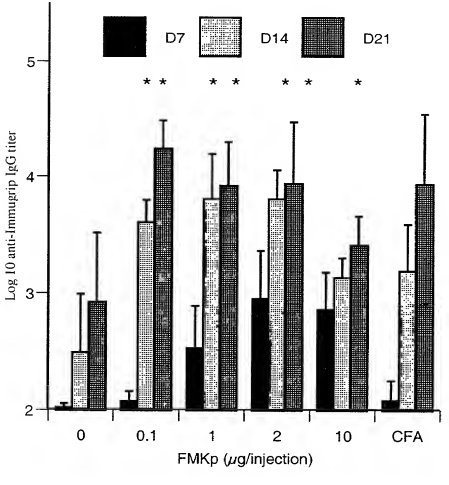

FIGURE 4

SEQUENCE LISTING

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65                  70                  75                  80
Val Asp Asn Gly Ala Phe Lys Ala Gln Gly Val Gln Leu Thr Ala Lys
                85                  90                  95
Leu Gly Tyr Pro Ile Thr Asp Asp Leu Asp Ile Tyr Thr Arg Leu Gly
            100                 105                 110
Gly Met Val Trp Arg Ala Asp Ser Lys Gly Asn Tyr Ala Ser Thr Gly
        115                 120                 125
Val Ser Arg Ser Glu His Asp Thr Gly Val Ser Pro Val Phe Ala Gly
    130                 135                 140
Gly Val Glu Trp Ala Val Thr Arg Asp Ile Ala Thr Arg Leu Glu Tyr
145                 150                 155                 160
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Gln Trp Val Asn Asn Ile Gly Asp Ala Gly Thr Val Gly Thr Arg Pro
                165                 170                 175
Asp Asn Gly Met Leu Ser Leu Gly Val Ser Tyr Arg Phe Gly Gln Glu
            180                 185                 190
Asp Ala Ala Pro Val Val Ala Pro Ala Pro Ala Pro Ala Pro Glu Val
        195                 200                 205
Ala Thr Lys His Phe Thr Leu Lys Ser Asp Val Leu Phe Asn Phe Asn
    210                 215                 220
Lys Ala Thr Leu Lys Pro Glu Gly Gln Gln Ala Leu Asp Gln Leu Tyr
225                 230                 235                 240
Thr Gln Leu Ser Asn Met Asp Pro Lys Asp Gly Ser Ala Val Val Leu
                245                 250                 255
Gly Tyr Thr Asp Arg Ile Gly Ser Glu Ala Tyr Asn Gln Gln Leu Ser
            260                 265                 270
Glu Lys Arg Ala Gln Ser Val Val Asp Tyr Leu Val Ala Lys Gly Ile
        275                 280                 285
Pro Ala Gly Lys Ile Ser Ala Arg Gly Met Gly Glu Ser Asn Pro Val
    290                 295                 300
Thr Gly Asn Thr Cys Asp Asn Val Lys Ala Arg Ala Ala Leu Ile Asp
305                 310                 315                 320
Cys Leu Ala Pro Asp Arg Arg Val Glu Ile Glu Val Lys Gly Tyr Lys
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Glu Val Val Thr Gln Pro Ala Gly
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ccg acc acc aaa cag cgt cag aac aaa ccg ccg aac aaa ccg aac aac   96
Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn Asn
            20                  25                  30
gat ttc cat ttc gaa gtg ttc aac ttc gtg ccg tgc agc atc tgc agc   144
Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Ser
        35                  40                  45
aac aac ccg acc tgc tgg gcg atc tgc aaa cgt atc ccg aac aaa aaa   192
Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys
    50                  55                  60
ccg ggc aaa aaa acc acg acc aaa ccg acc aaa aaa ccg acc ttc aaa   240
Pro Gly Lys Lys Thr Thr Thr Lys Pro Thr Lys Lys Pro Thr Phe Lys
65                  70                  75                  80
acc acc aaa aaa gat cat aaa ccg cag acc acc aaa ccg aaa gaa gtg   288
Thr Thr Lys Lys Asp His Lys Pro Gln Thr Thr Lys Pro Lys Glu Val
                85                  90                  95
ccg acc acc aaa ccg                                             303
Pro Thr Thr Lys Pro
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Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn Asn
            20                  25                  30
Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Ser
        35                  40                  45
Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys
    50                  55                  60
Pro Gly Lys Lys Thr Thr Thr Lys Pro Thr Lys Lys Pro Thr Phe Lys
65                  70                  75                  80
Thr Thr Lys Lys Asp His Lys Pro Gln Thr Thr Lys Pro Lys Glu Val
                85                  90                  95
Pro Thr Thr Lys Pro
            100
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Docket Number: PF 98 PCT SEQ

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below, next to my name, and

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Bacterial membrane fractions with adjuvant effect

the specification of which (check one of the following)

X is attached hereto

was filed on _____ as
Application Serial No. _____
And was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor(s) certificate having a filing date before that of the application on which priority is claimed:

Application Serial Number	Country	Filing Date (Day/Month/Year)	Priority Claimed (yes/no)
99 03153	FRANCE	15/March/1999	Yes

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

Docket Number: _____

(Application Serial No.)	(Filing Date)	(Status - patented, pending, abandoned)
PCT/FR00/00622	March 15, 2000	
(Application Serial No.)	(Filing Date)	(Status - patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status - patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following person registered to practice before the Patent and Trademark Office as my attorney with full power of substitution and revocation to prosecute this application and all divisions and continuations thereof and to transact all business in the Patent and Trademark Office connected therewith and request that all correspondence be sent to him at the mailing address hereafter given:

Name	G. PATRICK SAGE
Registration No.	37,710
Address	500 Columbia Plaza 350 East Michigan Ave. **Kalamazoo, MI 49007**

25666
PATENT TRADEMARK OFFICE

I further request that all telephone communications be directed to:

Name	G. PATRICK SAGE
Telephone	**616/382-0030**

Full Name of Sole/First Inventor: **LIBON Christine**

Inventor's Signature: _____ Date: 9.07.2001

Residence: **SAINT JULIEN EN GENEVOIS - FRANCE** (City, State) Citizenship: French (Country)

Post Office Address: **9 Avenue de Ternier – 74160 SAINT JULIEN EN GENEVOIS - FRANCE**

Full Name of Second/Joint Inventor: **CORVAIA Nathalie**

Inventor's Signature: _____ Date: 9.07.2001

Residence: **SAINT JULIEN EN GENEVOIS - FRANCE** (City, State) Citizenship: French (Country)

Post Office Address: **32 Rue des Chênes – 74160 SAINT JULIEN EN GENEVOIS - FRANCE**

Docket Number: _____

3-eD

Full Name of Third/Joint Inventor: **N'GUYEN Thien**

Inventor's Signature: _____ Date: 9.07.2001

Residence: **SAINT JULIEN EN GENEVOIS - FRANCE** Citizenship: French
(City, State) (Country)

PRX

Post Office Address: **7 Les Petits Lutins Lathoy – 74160 SAINT JULIEN EN GENEVOIS - FRANCE**

4-eD

Full Name of Fourth/Joint Inventor: **BECK Alain**

Inventor's Signature: ABL Date: 9.07.2001

Residence: **COLLONGES SOUS SALEVE - FRANCE** Citizenship: French
(City, State) (Country)

PRX

Post Office Address: **503 Route du poirier à l'âne – 74160 COLLONGES SOUS SALEVE - FRANCE**

5-eD

Full Name of Fifth/Joint Inventor: **BONNEFOY Jean-Yves**

Inventor's Signature: _____ Date: 9.07.2001

Residence: **LE SAPPEY - FRANCE** Citizenship: French
(City, State) (Country)

Post Office Address: **Les Noyers – 74350 LE SAPPEY - FRANCE** PRX

Full Name of Sixth/Joint Inventor:

Inventor's Signature: Date:

Residence: Citizenship:
(City, State) (Country)

Post Office Address: